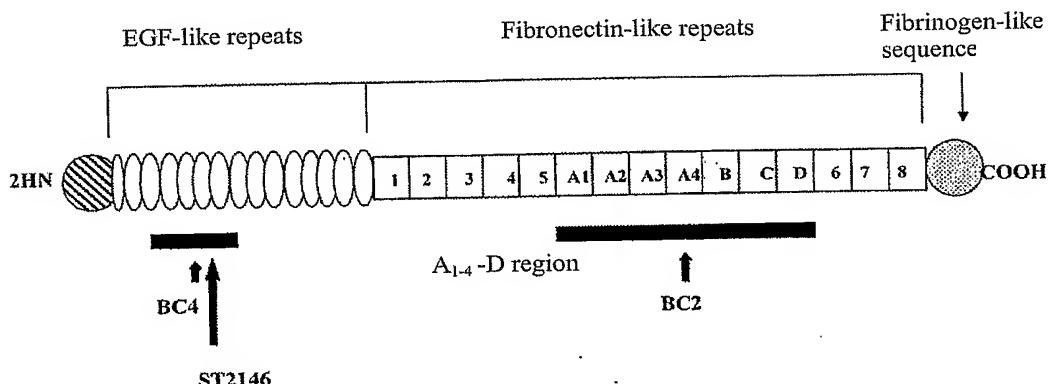


Figure 1

Schematic representation of human tenascin-C and of the strategy followed for the generation of BC2-like antibodies



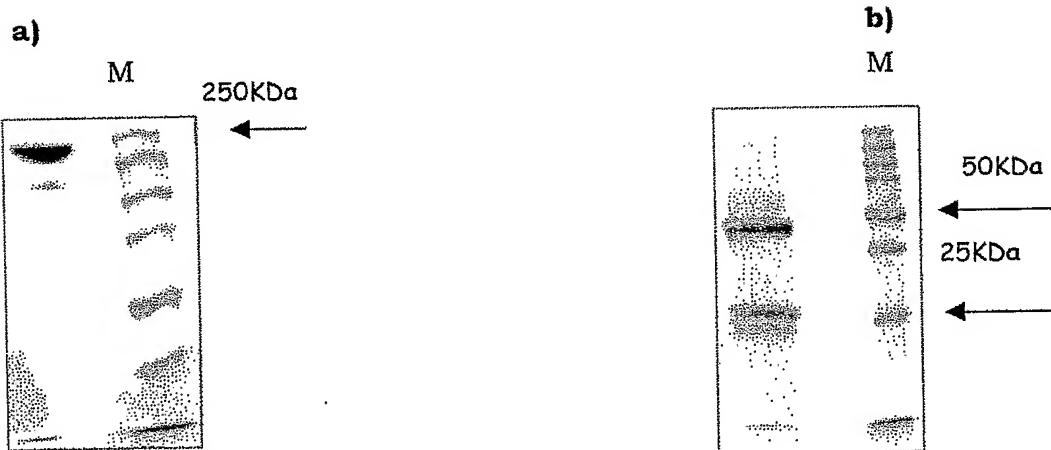
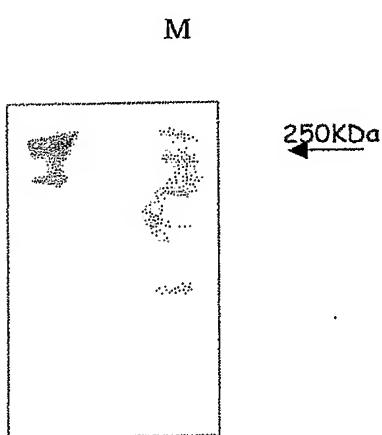
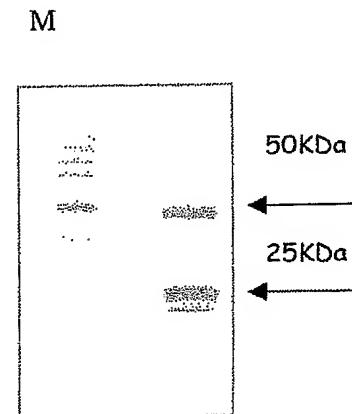
**Immunisation of Balb/c mice with Tn
(A₁₋₄-D) fragment**

**Fusion of spleen cells of immunised mice with
Sp2/0 Ag14 myeloma cells**

**Screening of culture supernatants of hybridomas
obtained on purified tenascin**

Figure 2

SDS-PAGE and Western Blot analysis of ST2485 antibody, in reducing (b, d) and non-reducing (a, c) conditions.

SDS-PAGE**Western Blot****c)****d)**

M: molecular weight standards

Figure 3

ST2485 antibody digestion with *Flavobacterium* Peptide-N-glycosidase enzyme (PNGase F).

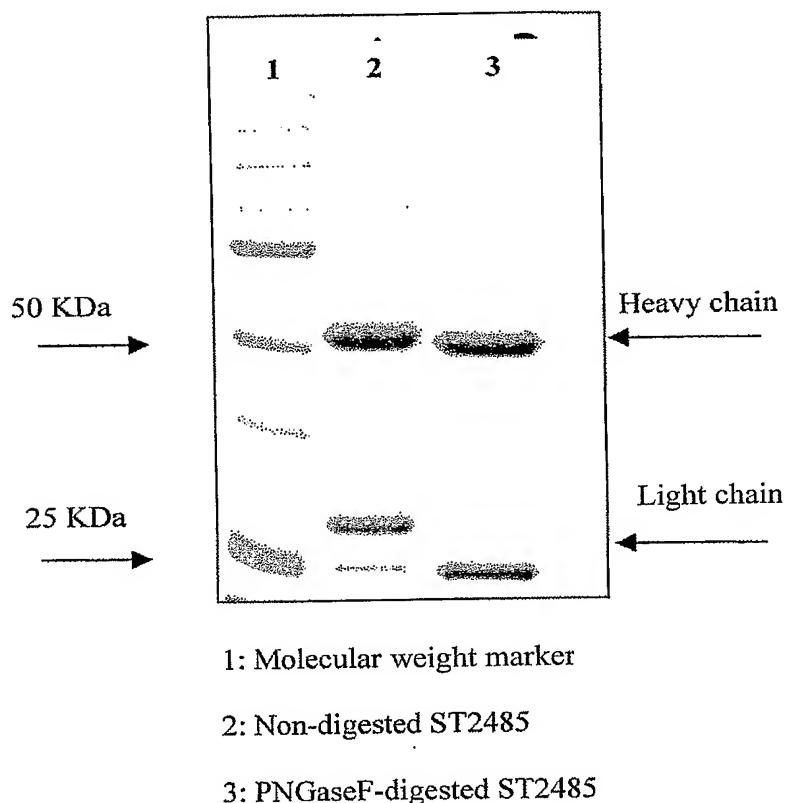


Figure 4

BC-2 and ST2485 antitenascin antibodies hydroxyapatite chromatography.

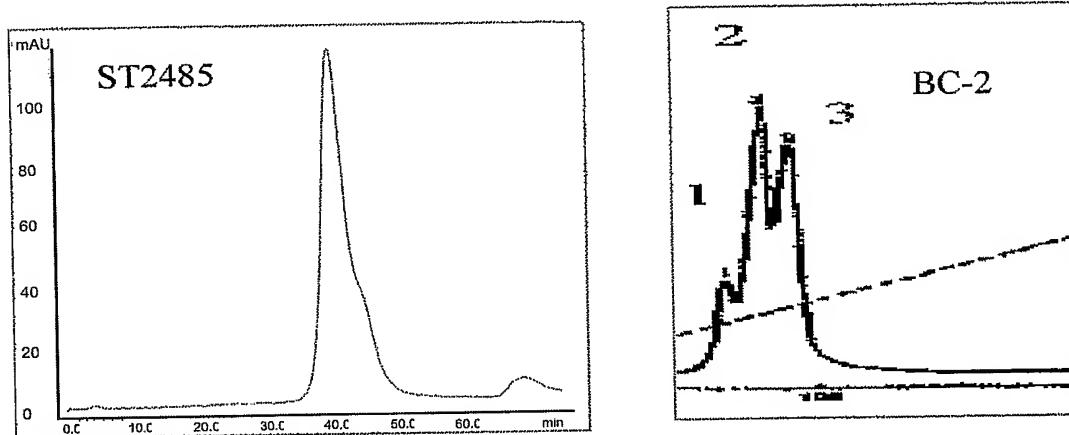
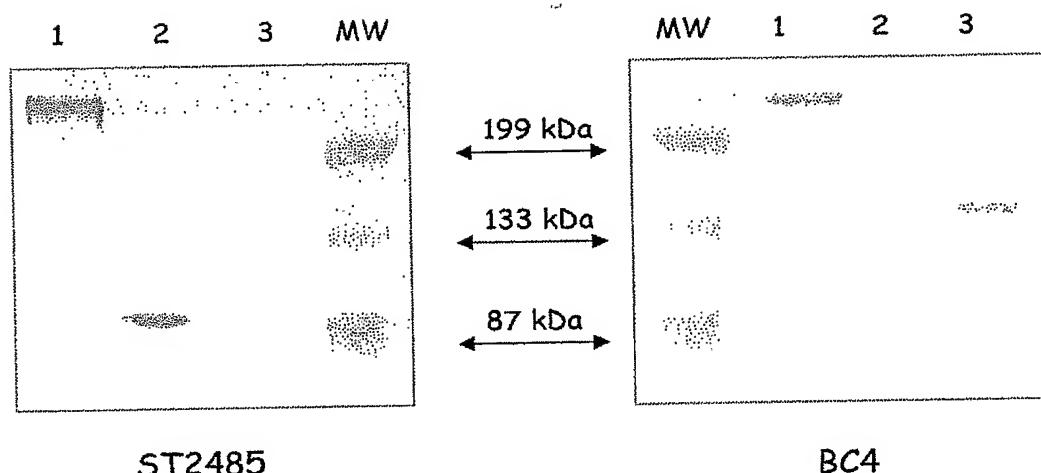


Figure 5**ST2485 antitenascin antibody Western Blot Analysis**

1: Tenascin-C

2: Tn A₍₁₋₄₎-D Fragment

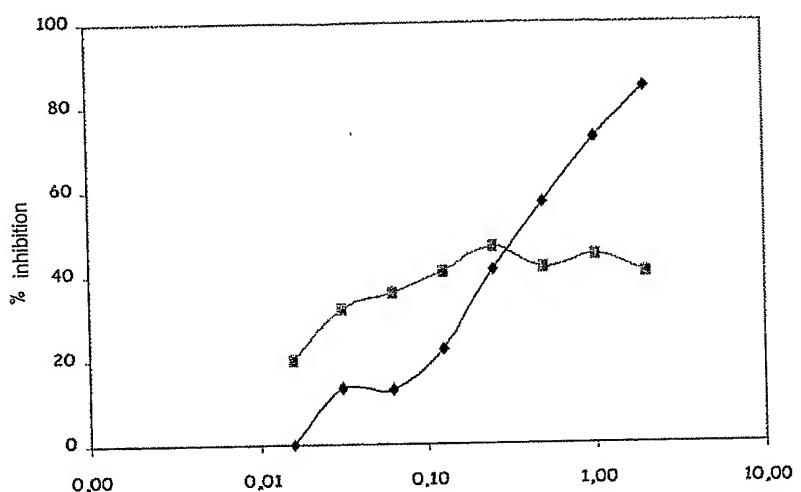
3: Tenascin EGF-like region recombinant fragment, containing the epitope recognized by BC-4 antibody.

MW: molecular weight standards

Figure 6

Competitive ELISA test between ST2485 and BC-2 for antigen binding.

a)



b)

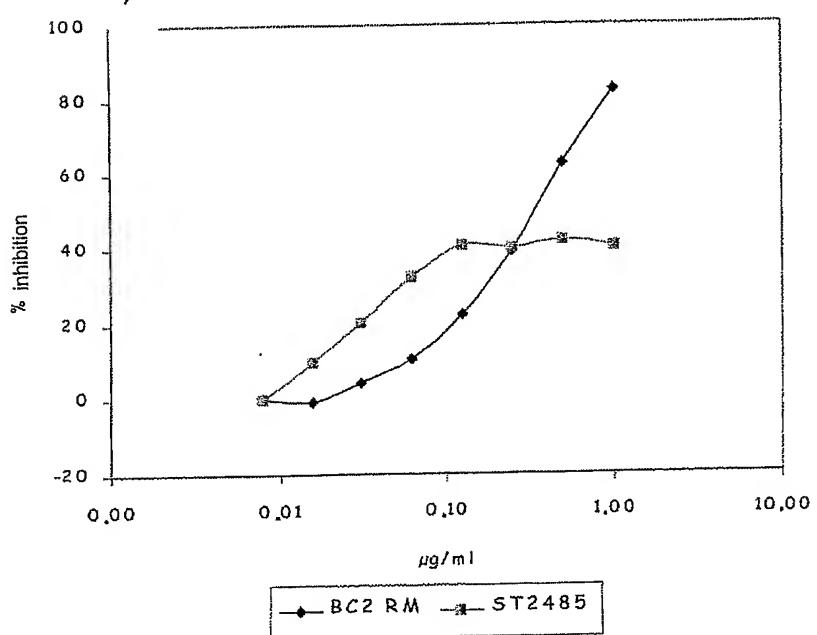
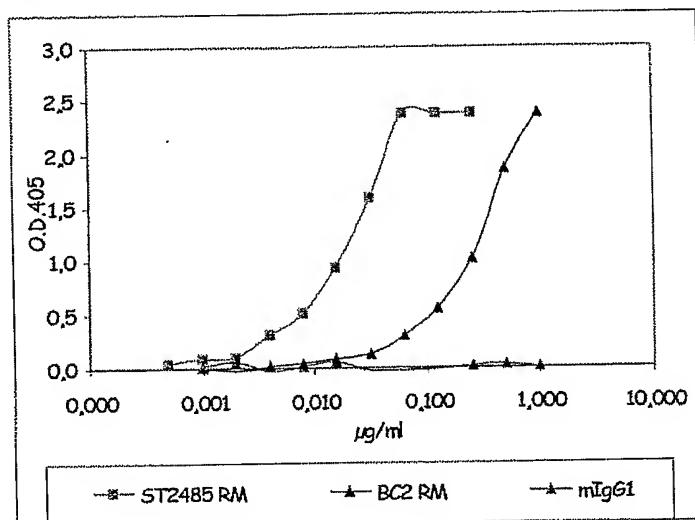


Figure 7

Immunoreactivity of ST2485 antibody in comparison with BC-2, on tenascin C (a) and on Tn A₍₁₋₄₎-D fragment (b).

a)



b)

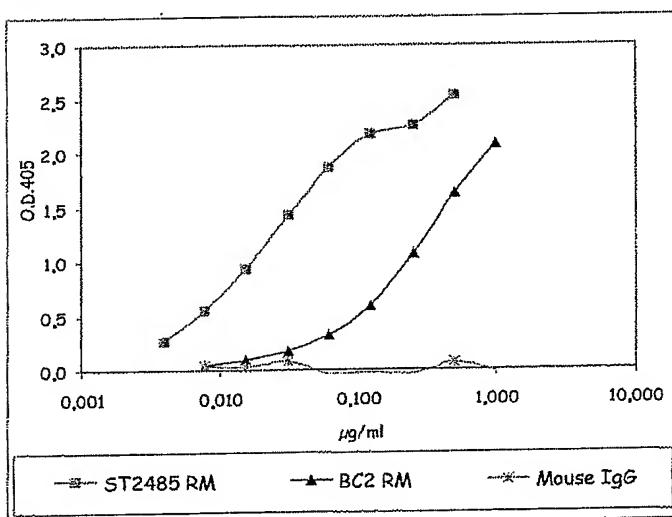


Figure 8

Immunoreactivity of ST2485 and BC-2 biotinylated and non-biotinylated antibodies, on tenascin-C (a) and on Tn A₍₁₋₄₎-D fragment (b).

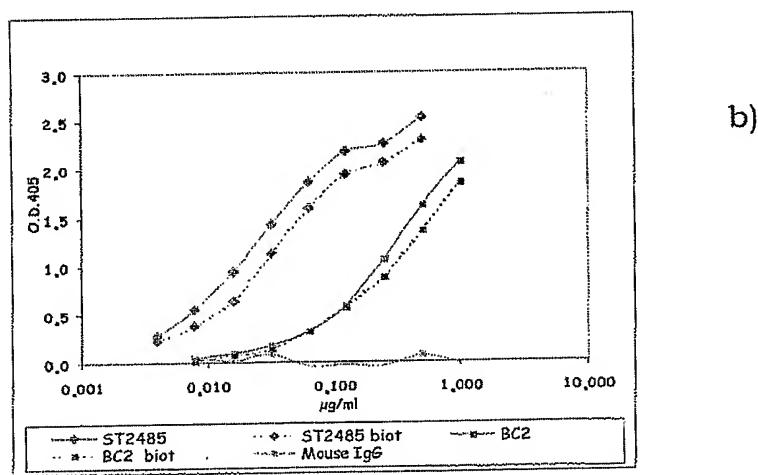
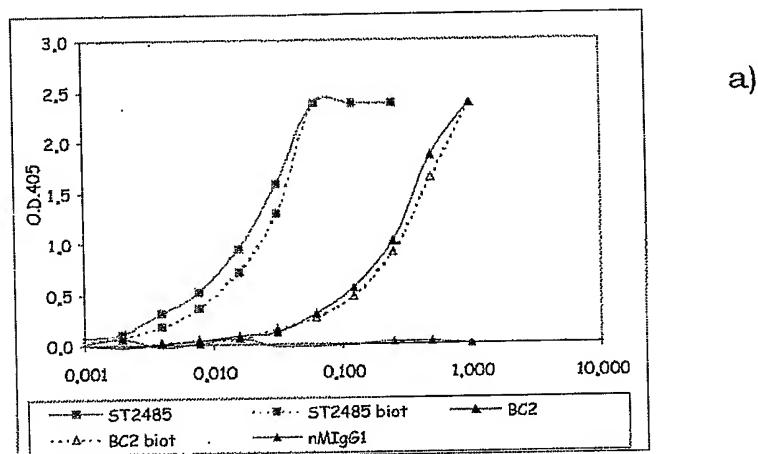


Figure 9

Cross-reactivity of ST2485 antibody with murine tenascin.

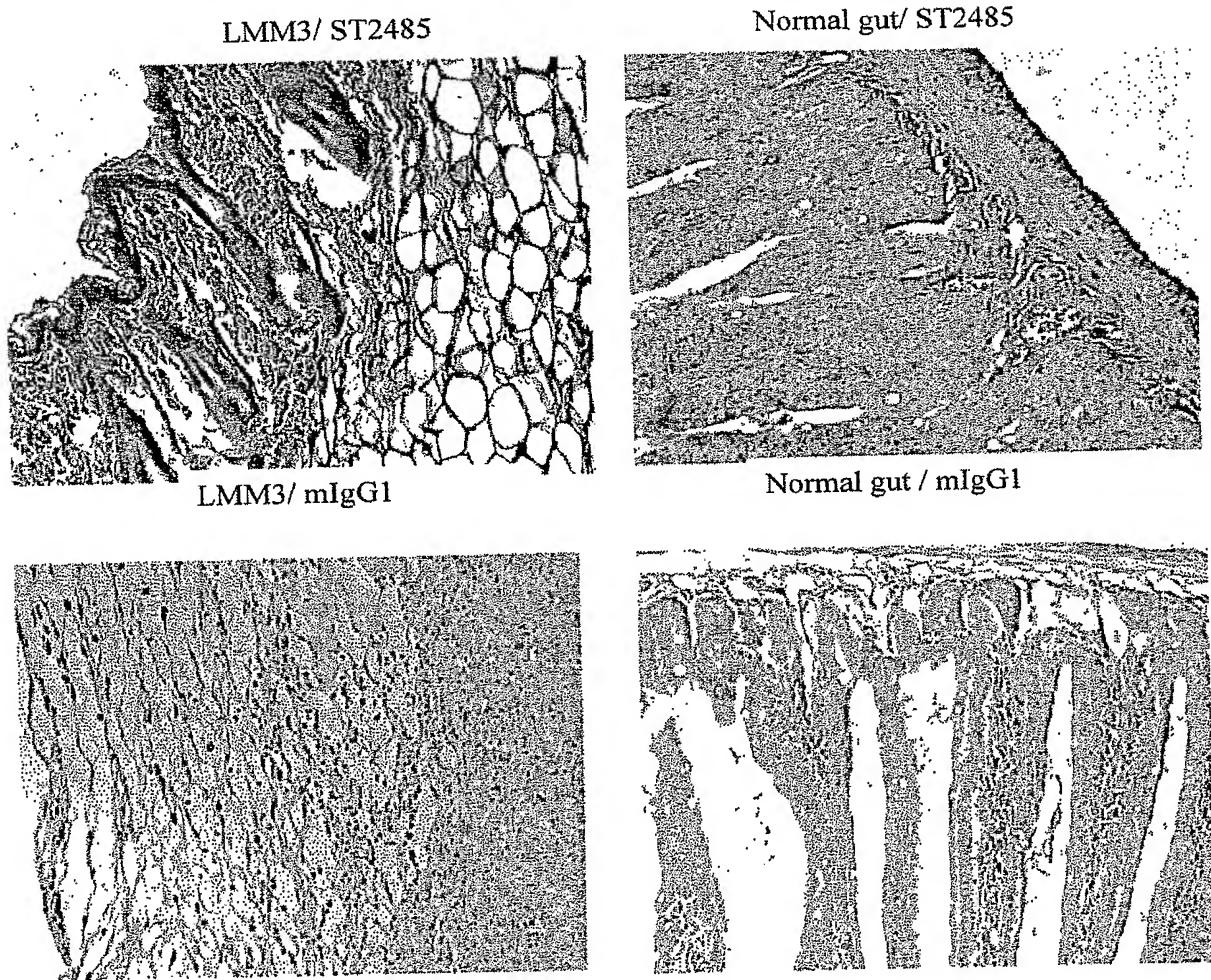


Figure 10

Biotinylated ST2485 and BC-2 antibodies biodistribution study protocol in human tenascin-expressing tumor-implanted nude mice.

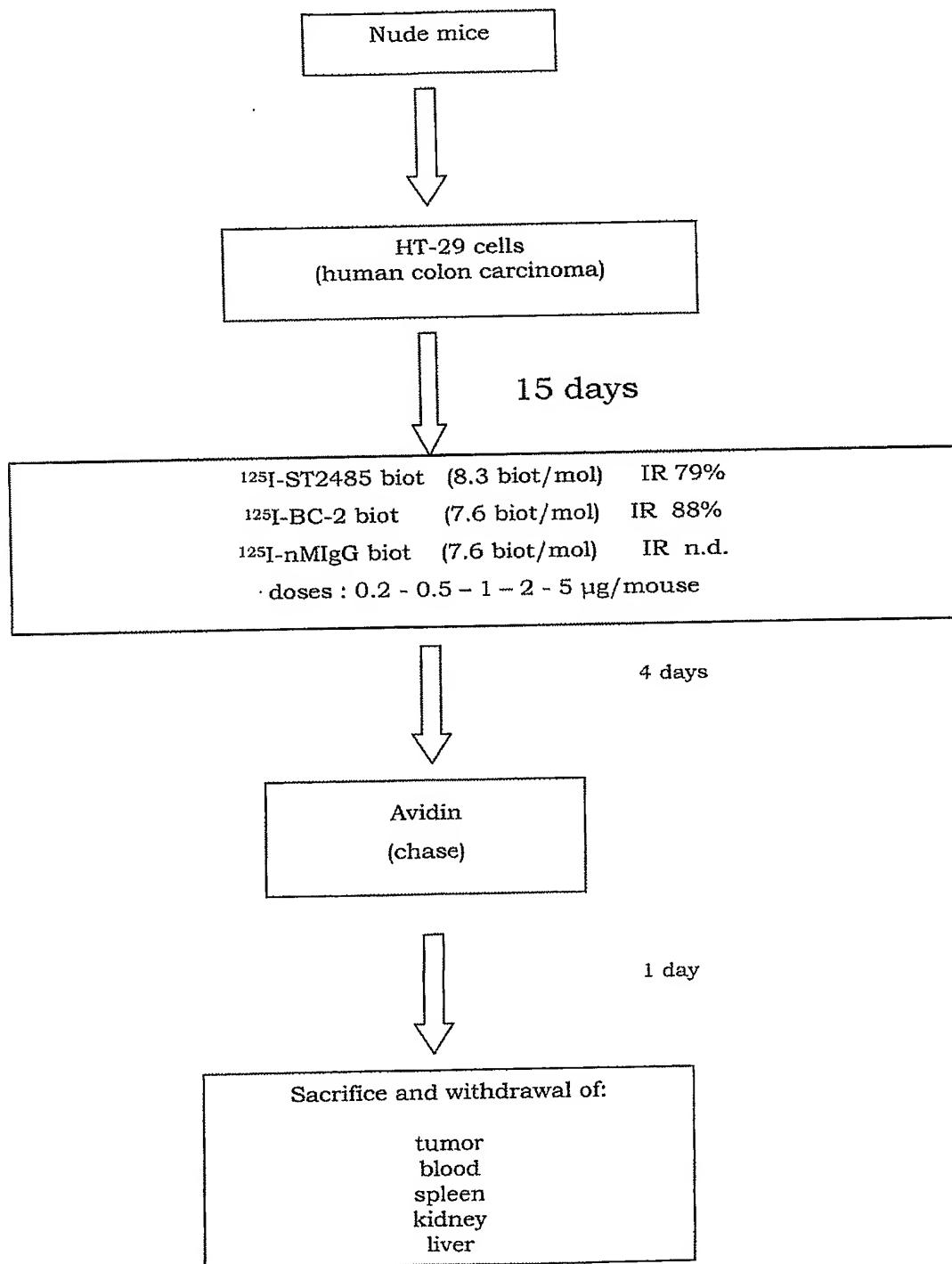


Figure 11

Biotinylated ST2485 and BC-2 biodistribution in human tenascin-expressing tumor-transplanted nude mice. The antibody amount is expressed as percent of the injected dose per tissue gram (% I.D./gr).

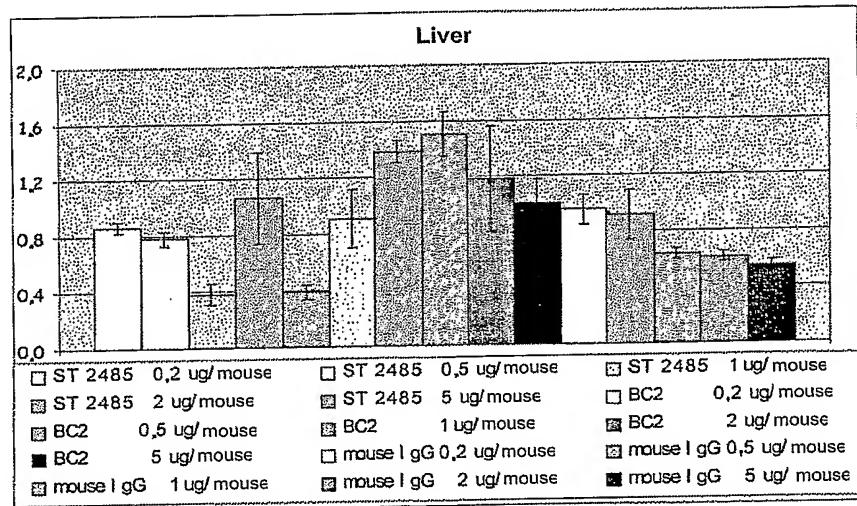
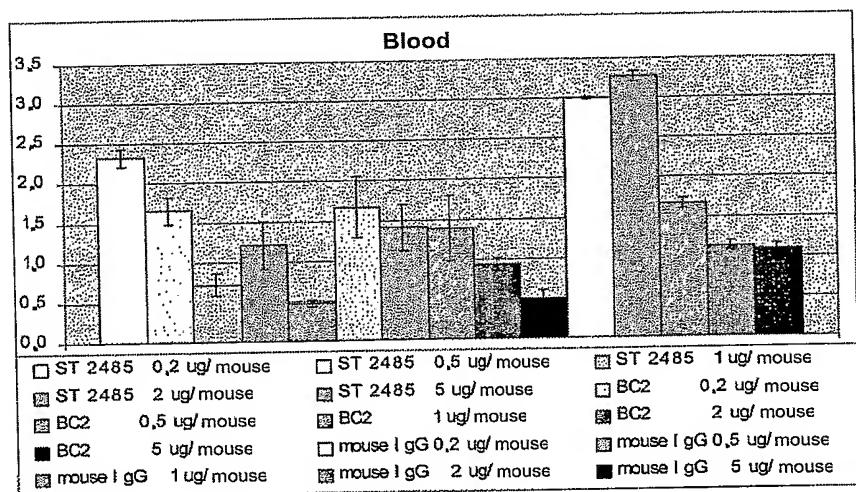


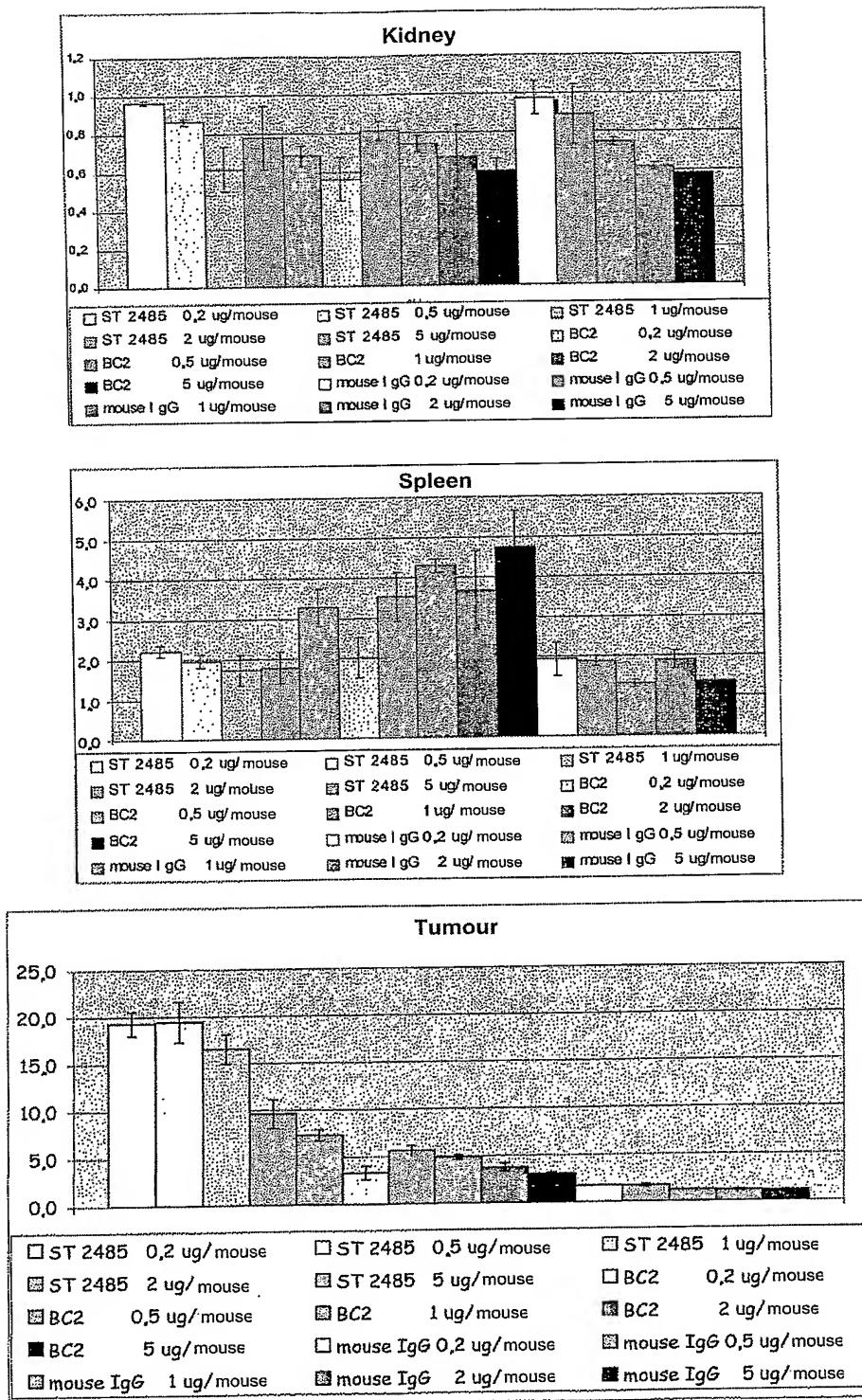
Figure 11a

Figure 12

Biotinylated ST2485 and BC-2 biodistribution in nude mice: tumor/non tumor ratio.

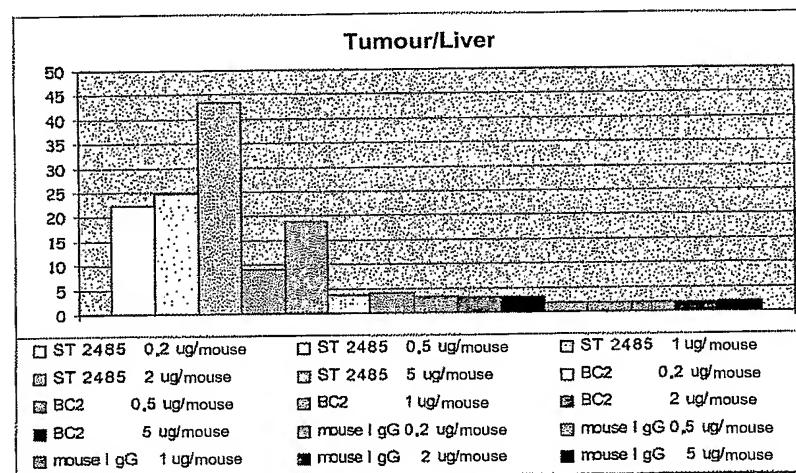
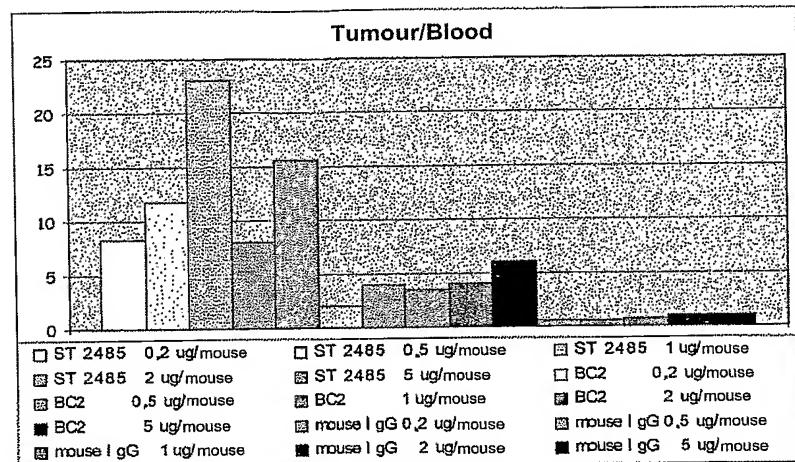


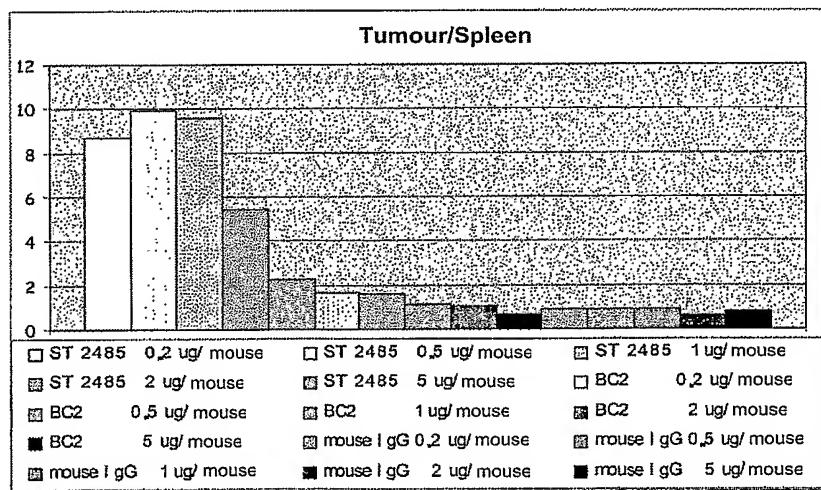
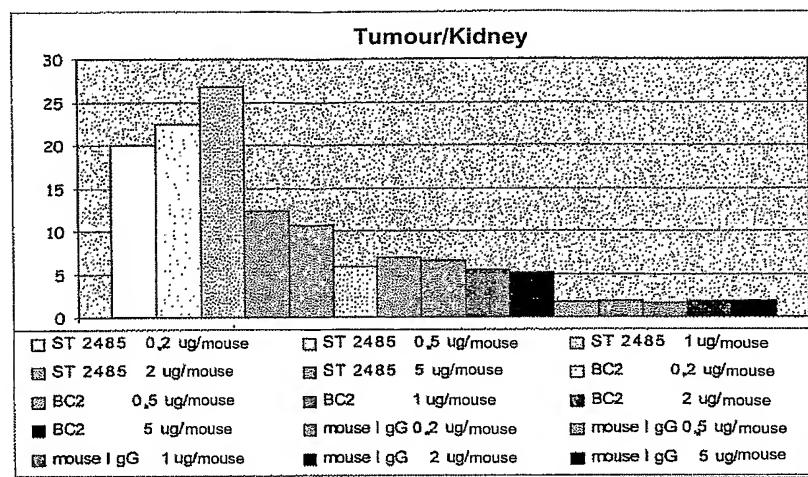
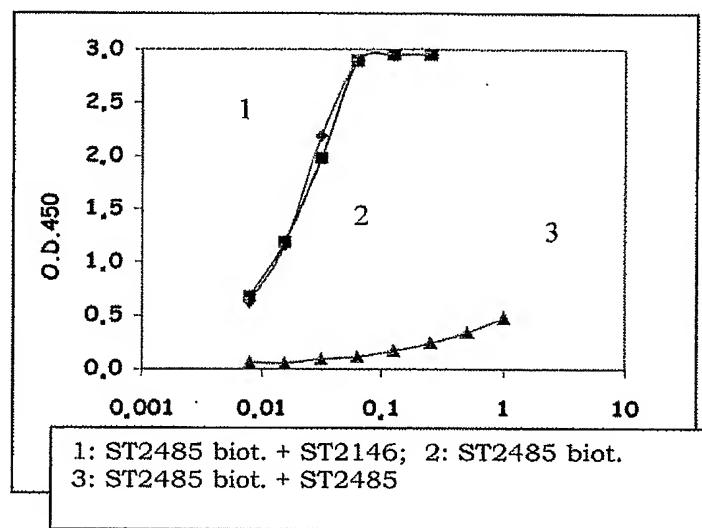
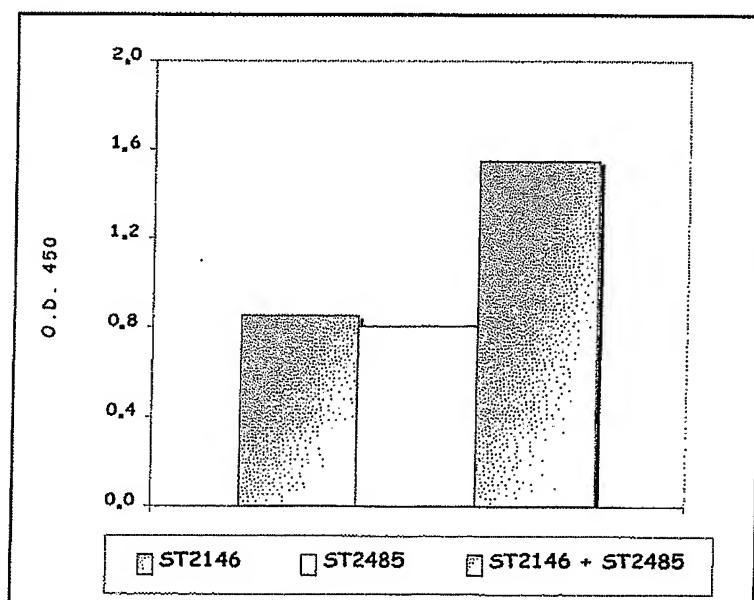
Figure 12a

Figure 13

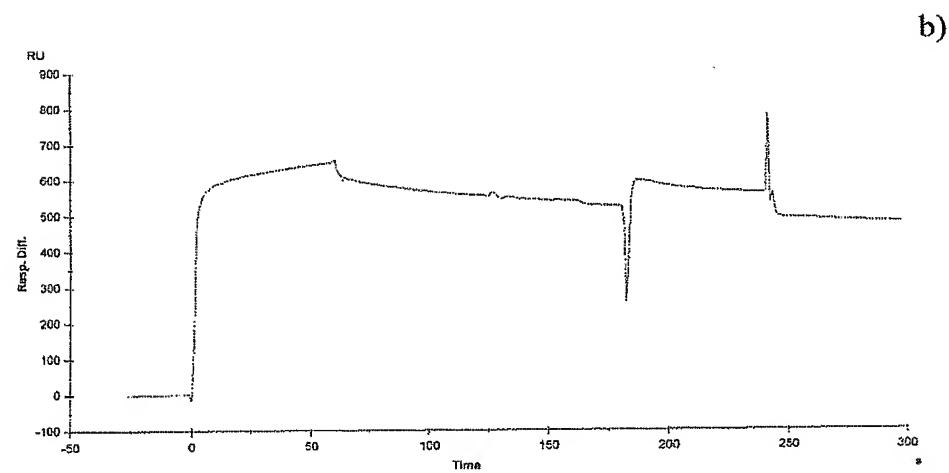
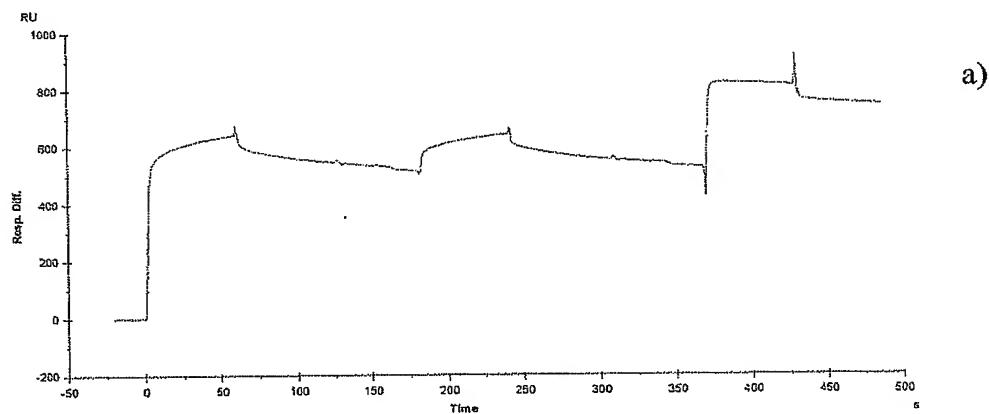
Interference (a) and additivity (b) ST2485 and ST2146 antitenascin antibodies *in vitro* evaluation by ELISA test.

a) Interference**b) Additivity**

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Figure 14

Antibodies ST2485 and ST2146 tenascin binding *in vitro* additivity by BIACore



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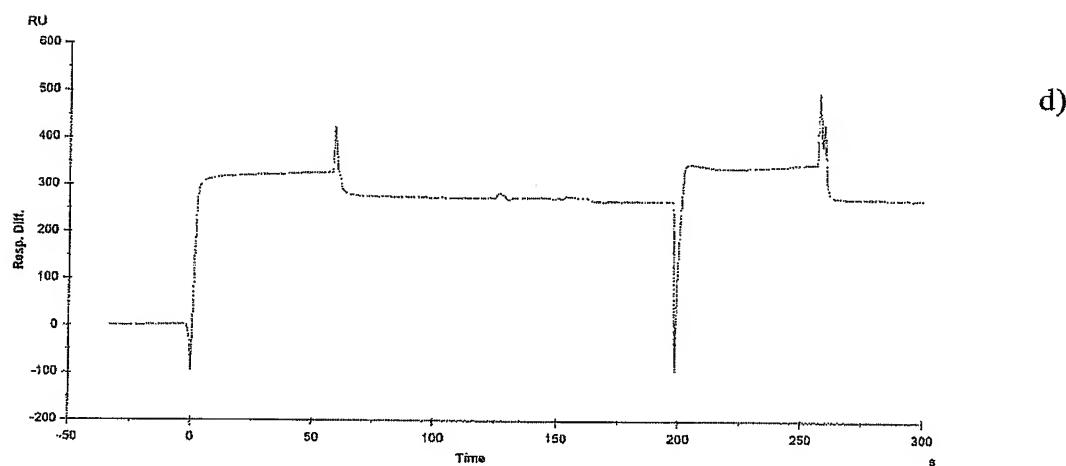
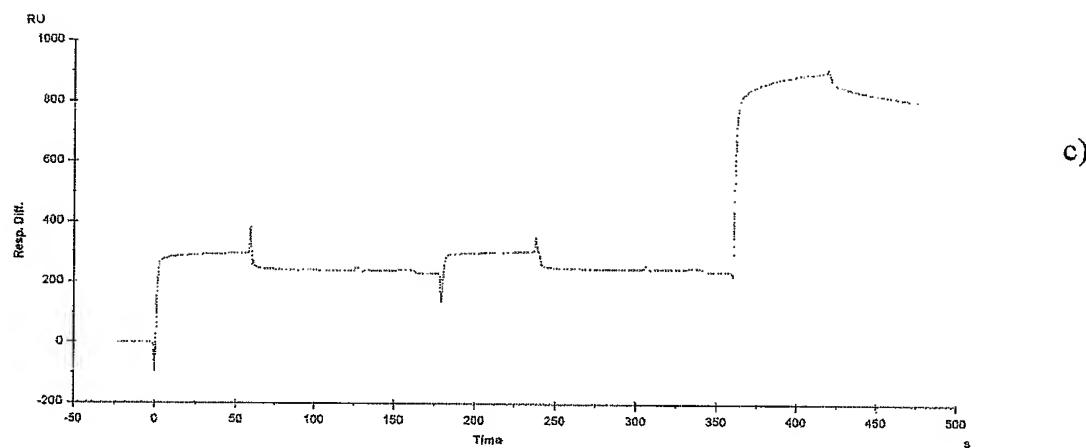
Figure 14a

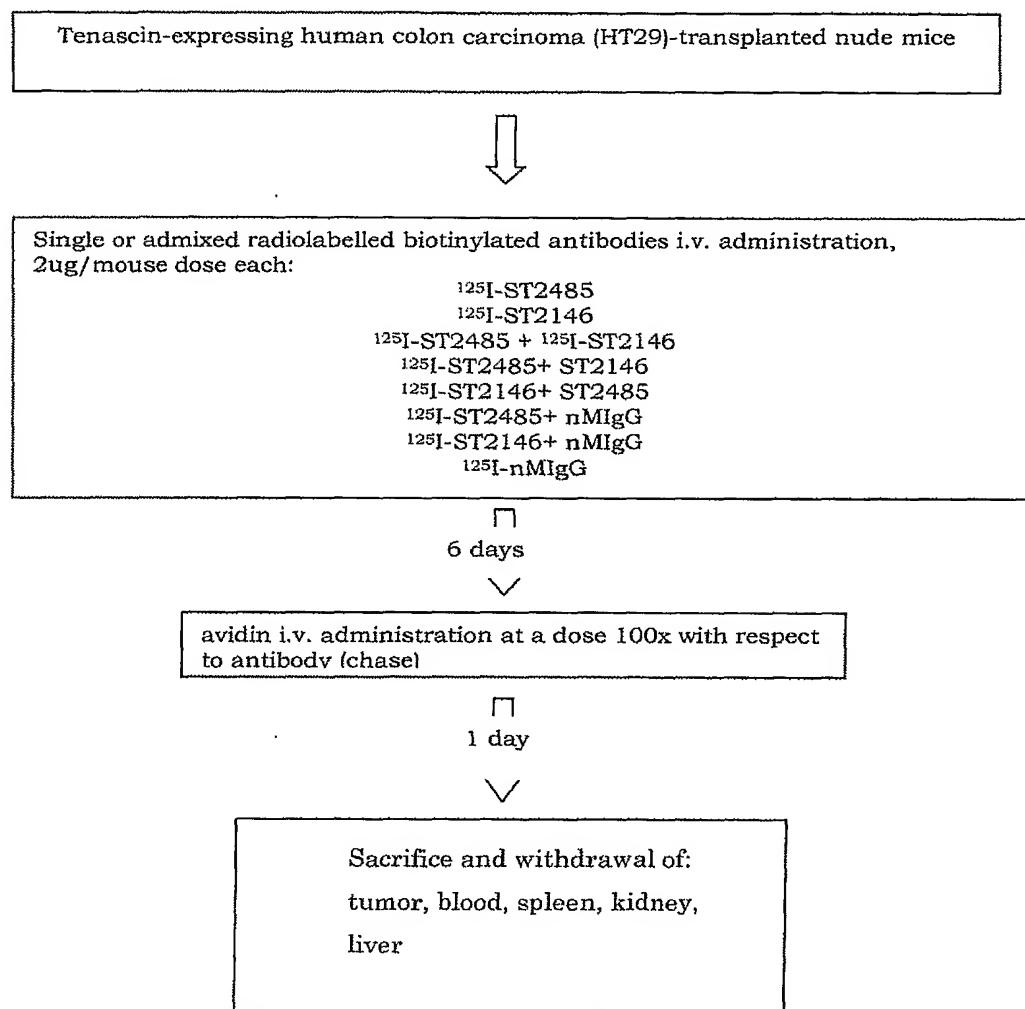
Figure 15Schematic representation of *in vivo* additivity study in animal model

Figure 16

ST2485 e ST2146 antibodies additivity in animal model; tumor seat localization.

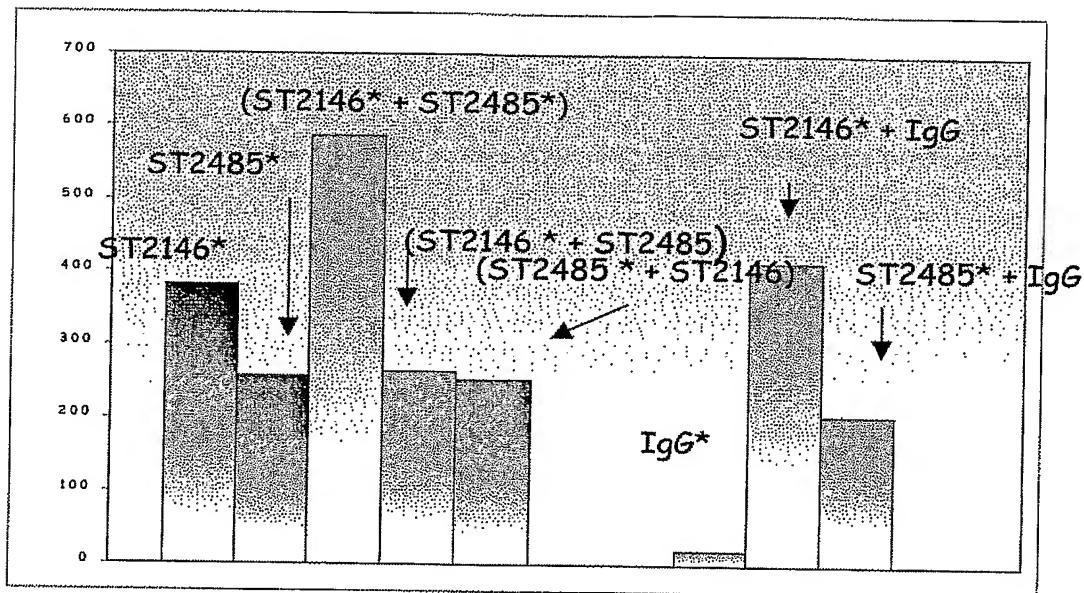


Figure 17

SEQID 1 sequence of ST2485 kappa light chain variable region (VL).

Signal peptide

~~ATGGATTTCAGTGCAGATTTAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTC~~**CCAGGGACAAA**
Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser Val Ile Met Ser Arg Gly Gln

TTGTTCTCTCCCAGTCTCCAGCAATCCTGTCATCTCCAGGGAGAAGGTCACAATGACTTGC
Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys

CDR1 ↓ N-glycosylation
~~AGGGCCAACTCAAGTGTACGTTCATGCACTGGTACCGAGCAGAAGCCAGGATCCTCCCCAAACC~~
Arg Ala Asn Ser Ser Val Arg Phe Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys

CDR2
~~CTGGATTATGCGCTTCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGTCTGG~~
Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly

CDR3
~~GACCTCTTATTCTGTACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGC~~
Ser Gly Thr Ser Tyr Ser Val Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln

~~ATGGATTTCAGTGCAGATTTAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTC~~**CCAGGGACAAA**
Gln Trp Ser Ser Asn Ser Pro Arg Thr Phe Gly Gly Thr Lys Val Glu Ile Arg Arg Ala

Figure 18

SEQID 2 sequence of ST2485 gamma heavy chain variable region (VH)

Signal peptide

*ATGGGATGGAGCTGGATCCTTCTCTCCCTCCTGTCAGGAAC*CTGCAGGTGTCCTCAGGTCCAGCTG
Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly Val His Ser Glu Val Gln Leu

CAACAGTCTGGACCTGAGCTGGTGAAGCCTGGAGCTTCATGAAGATTCTGCAAGGCTTCTGG
Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Met Lys Ile Ser Cys Lys Ala Ser

CDR1

TTACTCATTCACTGGGACCATGAAACPGGGTGAAGCAGAGCCATGAAAGAACCTTGAATGGA
Gly Tyr Ser Phe Thr Gly Tyr Ile Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp

CDR2

TTGGACTTAAATGCTACAAATGGTGGTACTTACCTACAAACCAGAAGTCAAGGGCAAGGCCACA
Ile Gly Leu Ile Asn Pro His Asn Gly Gly Thr Thr Asn Gln Lys Phe Lys Gly Lys Ala Thr

TTAACTGTAGACAAGTCATCCAACACAGCCTACATGGAGCTCCTCAGTCTGACATCTGAGGACTC
Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp

CDR3

TGCAGTCTATTFACTGTACAAGACCCGGGGGTTACTACTGGTTCTTCGATGTCTGGGGCGCAGGGGA
Ser Ala Val Tyr Tyr Cys Thr Arg Pro Gly Gly Tyr Tyr Trp Phe Phe Asp Val Trp Gly Ala Gly

CCACGGTCACCGTCTCCTCA
Thr Thr Val Thr Val Ser Ser